

flasks should be minimal, since excessive drying of the paper can lead to erratic elutions.

(vii) *Elution.* To each flask add 50 milliliters of 0.05*N* methanolic hydrochloric acid and agitate on a reciprocating shaker for 1 hour. Decant the contents of each flask into another flask by pouring through a small funnel fitted with a glass wool plug.

(viii) *Doxycycline standard solution for direct measurement of absorbance.* Pipette a 0.100-milliliter aliquot of the doxycycline standard solution into

each of three 125-milliliter Erlenmeyer flasks. Add 50 milliliters of 0.05*N* methanolic hydrochloric acid to each of these flasks.

(ix) *Absorbance measurement.* Using a suitable spectrophotometer and 0.05*N* methanolic hydrochloric acid as the reference solvent, determine the absorbance of each eluate and of each doxycycline standard solution at the absorption maximum at about 349 nanometers.

(x) *Calculation of percent doxycycline in samples.* Calculate as follows:

$$\text{Percent doxycycline} = \frac{(A_u - A_b)(W_s)}{(A_s - A_b)(W_u)} \times \text{Doxycycline content of the working standard}$$

where:

$A_u$ =Absorbance of the eluate from the main doxycycline band of the sample sheet.

$A_s$ =Absorbance of the eluate from the main doxycycline band on the standard sheet.

$A_b$ =Absorbance of the eluate from the area of the blank sheet corresponding to the area of the doxycycline band of the standard sheet.

$W_u$ =Weight in milligrams of sample.

$W_s$ =Weight in milligrams of doxycycline working standard.

(xi) *Recovery of the doxycycline standard from the chromatogram.* As follows:

$$\text{Percent recovery} = \frac{A_s - A_b}{A_p} \times \frac{100}{F}$$

where:

$A_p$ =Absorbance of the doxycycline standard solution described in paragraph (b)(5)(viii) of this section.

$F$ =The fractional purity of doxycycline standard solution described in paragraph (b)(5)(xii) of this section.

If the recovery of the doxycycline standard from the chromatogram is less than 95 percent, repeat the chromatogram.

(xii) *Determination of the fractional purity of the doxycycline working standard.* Determine  $F$  by means of the following equation:

$$F = 1 - \frac{A_c - A_{cb}}{A_c - A_{cb} + A_s - A_b}$$

where:

$A_c$ =Absorbance of the eluate from sections of the standard chromatogram containing nondoxycycline 349 nanometers-absorbing contaminants.

$A_{cb}$ =Absorbance of the eluates from the sections of the blank sheets corresponding to those sections of the nondoxycycline-absorbing contaminants of the standard sheets.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 50 FR 19920, May 13, 1985]

#### § 446.20a Sterile doxycycline hyclate.

(a) *Requirements for certification—(1) Standards of identity, strength, equality, and purity.* Sterile doxycycline hyclate is [4S - (4 $\alpha$ ,4 $\alpha$ ,5 $\alpha$ ,5 $\alpha$ ,6 $\alpha$  12 $\alpha$ )] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11,12a - octahydro - 3,5,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphthacene-carboxamide hydrochloride hemiethanolate hemihydrate. It is so purified and dried that:

(i) Its potency is not less than 800 nor more than 920 micrograms of doxycycline per milligram on an "as is" basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) It contains no depressor substances.

(vi) Its moisture content is not less than 1.4 nor more than 2.75 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.0 nor more than 3.0.

(viii) It contains not less than 82 nor more than 90 percent doxycycline on an "as is" basis.

(ix) It gives a positive identity test for doxycycline hyclate.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this subchapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this subchapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, depressor substances, moisture, pH, doxycycline content, identity, and crystallinity.

(ii) Samples required:

(a) For all tests except sterility: 12 packages, each containing approximately 300 milligrams.

(b) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1N hydrochloric acid to obtain a concentration of 1,000 micrograms of doxycycline per milliliter (estimated). Further dilute with sterile distilled water to the reference concentration of 0.100 microgram of doxycycline per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this subchapter, using the method described in paragraph (e)(1) of that section, except use diluting fluid D in lieu of diluting fluid A.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this subchapter, using a solution containing 7.5 milligrams of doxycycline per milliliter.

(4) [Reserved]

(5) *Depressor substances.* Proceed as directed in § 436.35 of this subchapter.

(6) *Moisture.* Proceed as directed in § 436.201 of this subchapter.

(7) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing the equivalent of 10 milligrams of doxycycline per milliliter.

(8) *Doxycycline content.* Proceed as directed in § 446.20(b)(5).

(9) *Identity.* Proceed as directed in § 436.211 of this subchapter, using the 0.25 potassium bromide mixture described in paragraph (b)(1) of that section.

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this subchapter.

[39 FR 19076, May 30, 1974, as amended at 43 FR 11155, Mar. 17, 1978; 46 FR 60568, Dec. 11, 1981; 50 FR 19920, May 13, 1985]

#### § 446.21 Doxycycline monohydrate.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Doxycycline monohydrate is [4S - (4 $\alpha$ ,4a $\alpha$ ,5 $\alpha$ ,5a $\alpha$ ,6 $\alpha$ ,12a $\alpha$ )] - 4 - (dimethylamino) - 1,4,4a,5,5a,6,11, - 12a - octahydro - 3,5,10,12,12a - pentahydroxy - 6 - methyl - 1,11 - dioxo - 2 - naphtha - cenecarboxamide monohydrate. It is so purified and dried that:

(i) Its potency is not less than 880 micrograms nor more than 980 micrograms of doxycycline per milligram on an "as is" basis.

(ii) [Reserved]

(iii) Its moisture content is not less than 3.6 percent nor more than 4.6 percent.

(iv) Its pH in an aqueous suspension containing the equivalent of 10 milligrams of doxycycline per milliliter is not less than 5.0 nor more than 6.5.

(v) It contains not less than 90 percent nor more than 98 percent doxycycline on an "as is" basis.

(vi) It gives a positive identity test for doxycycline monohydrate.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, doxycycline content, identity, and crystallinity.